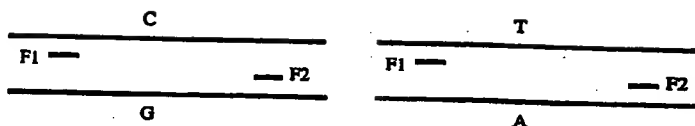
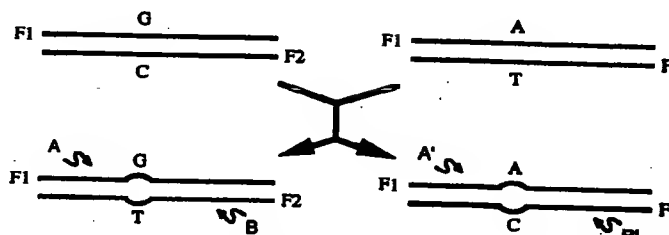


Mismatch scanning Assay. (Endo V / DNA Ligase)

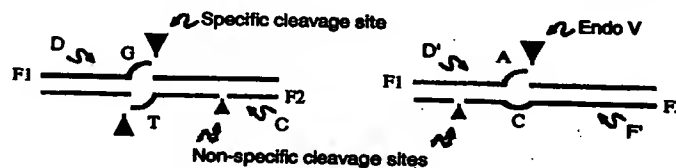
1. PCR amplify gene using primers with different fluorescent labels and *Taq* DNA polymerase.



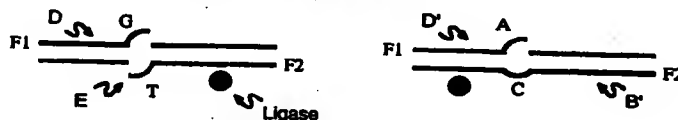
2. Denature and reanneal PCR products to form heteroduplexed DNA. (Homoduplexed products not shown).



3. Preferentially nick DNA one base to the 3' side of mismatches using thermostable Endonuclease V.



4. Add thermostable ligase to re-seal background nicks at perfect match regions.



5. Separate fluorescent products on a DNA sequencer (using length standards) to approximate site of mismatch.

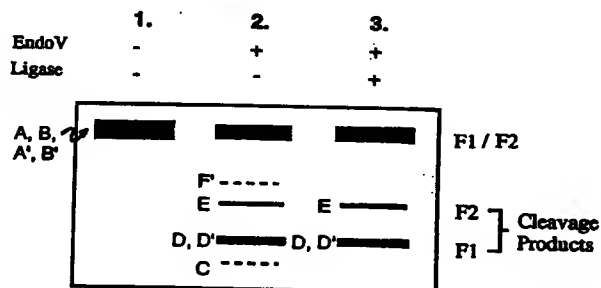


Figure 1

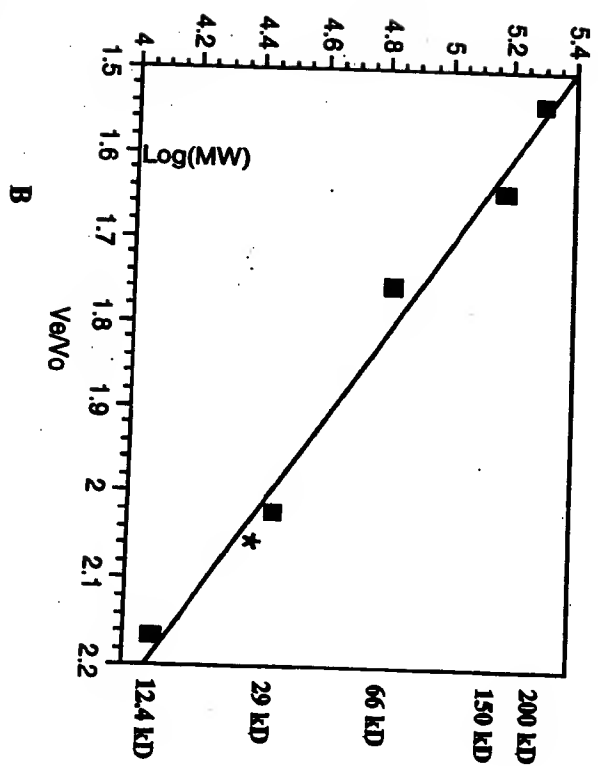
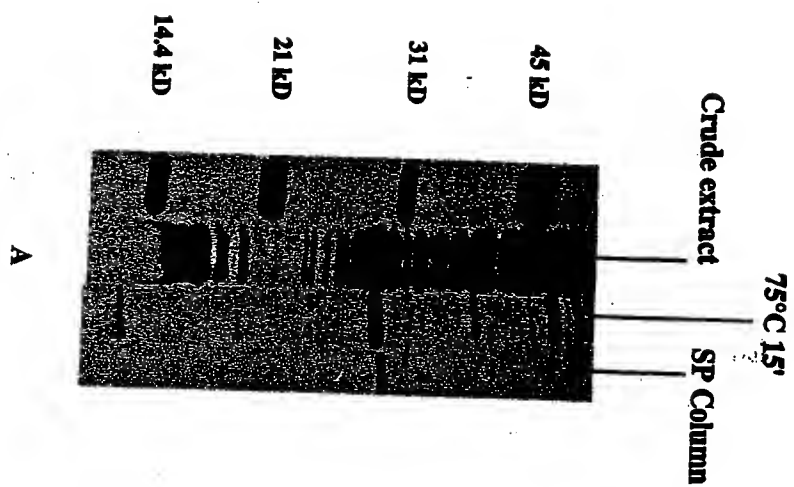
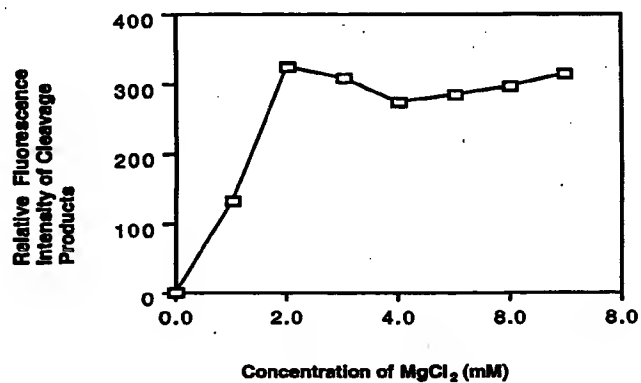


Figure 2

A



B

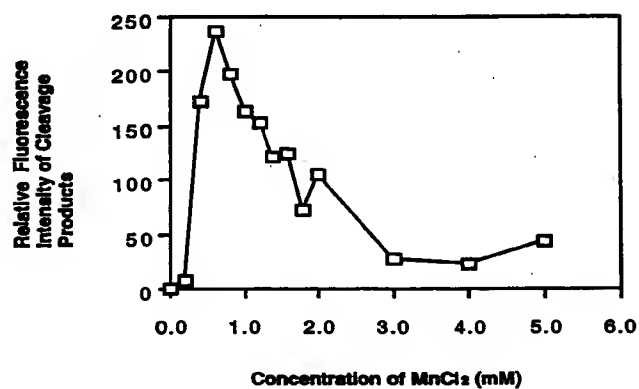


Figure 4

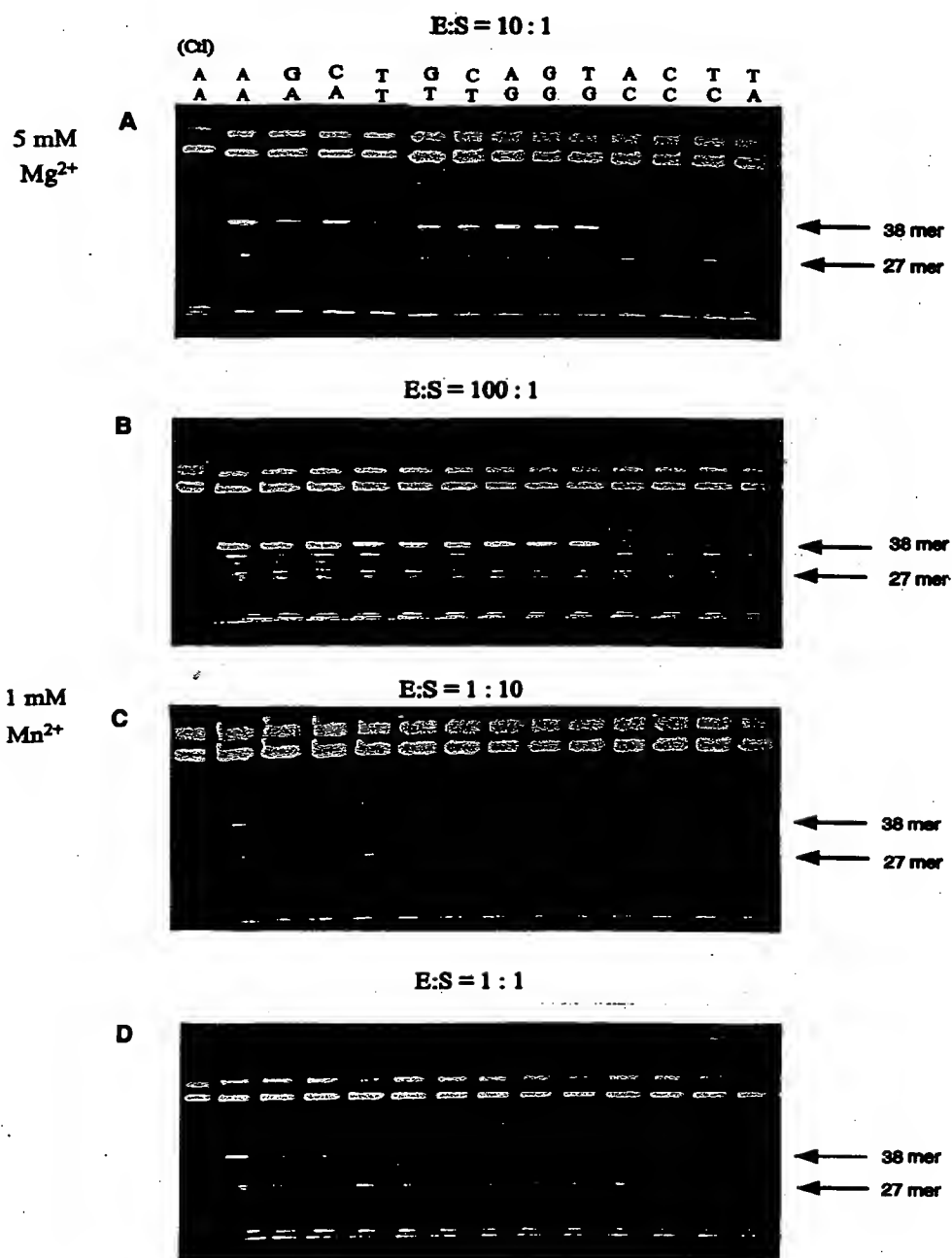


Figure 5

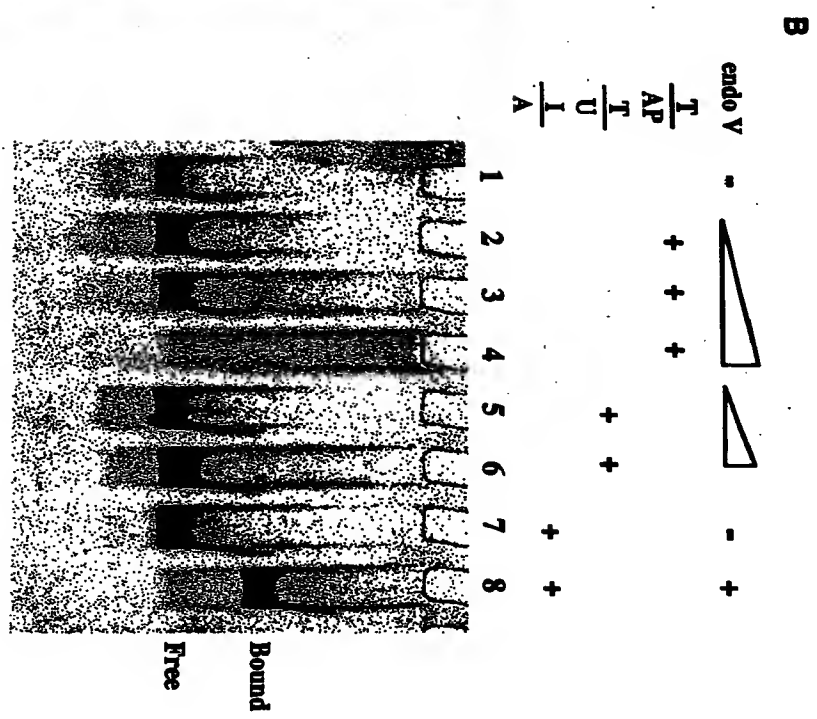
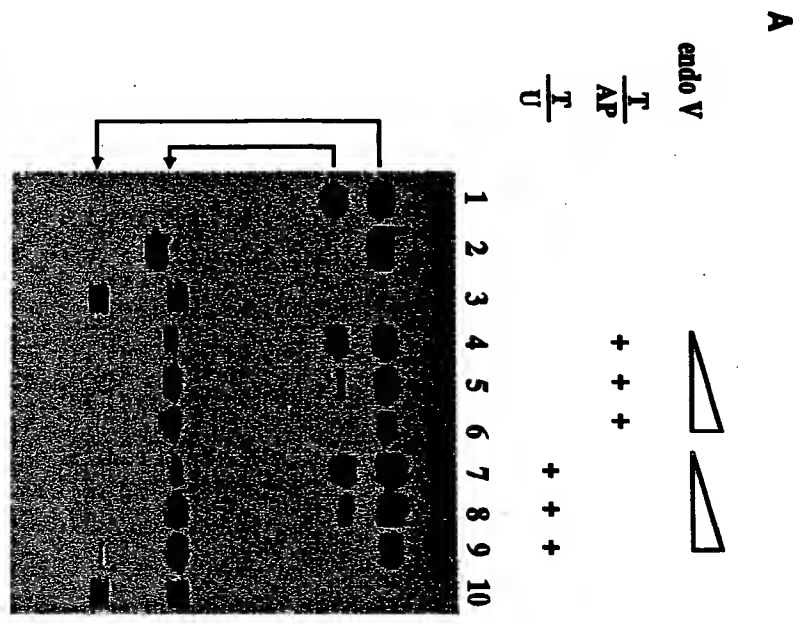


Figure 6

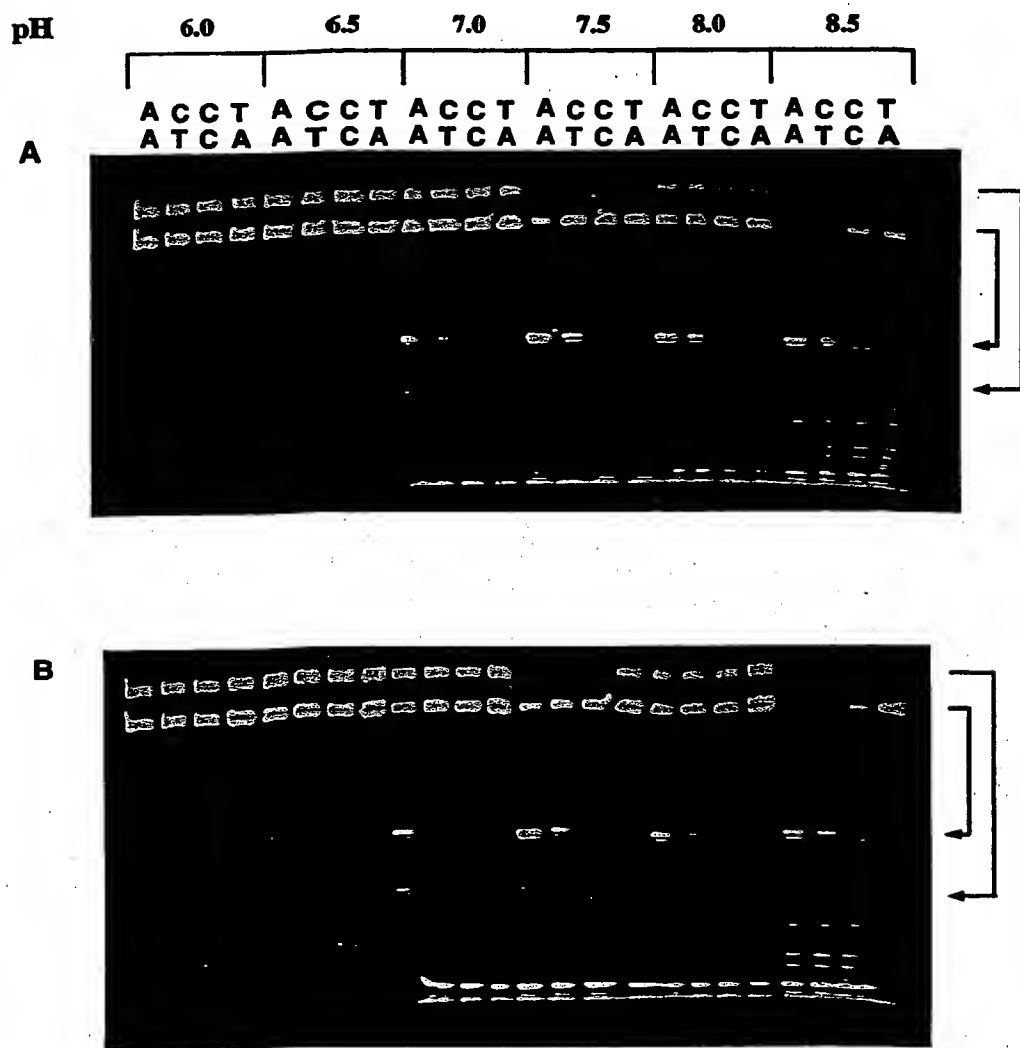


Figure 7

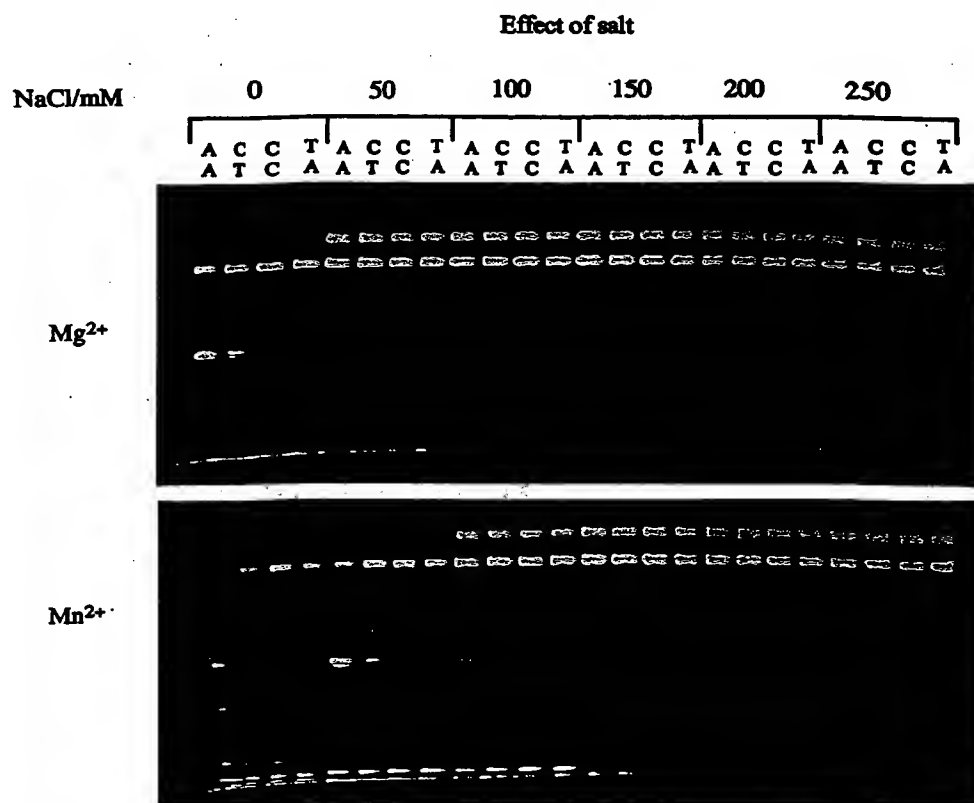


Figure 8

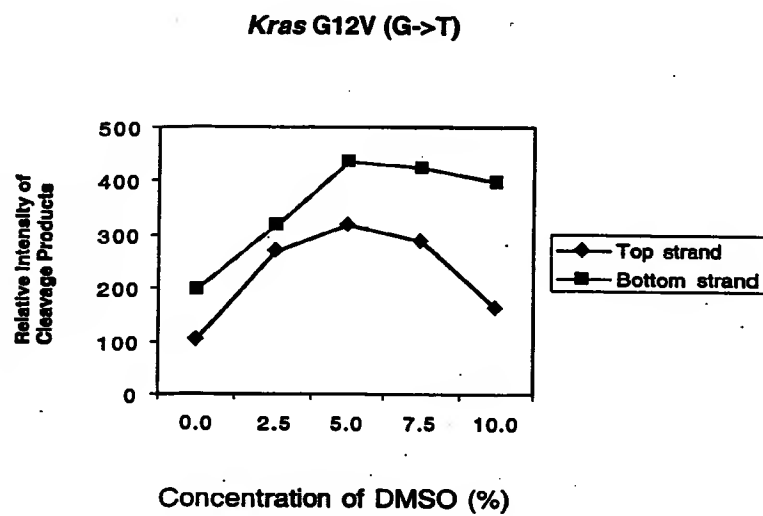
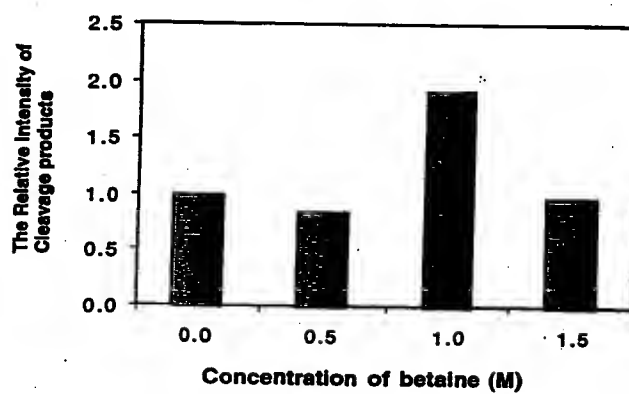


Figure 9

A

APC I1307K(T->A)



B

Kras G12V (G->T)

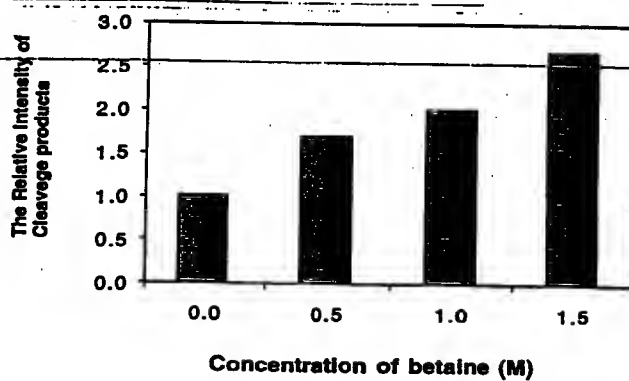
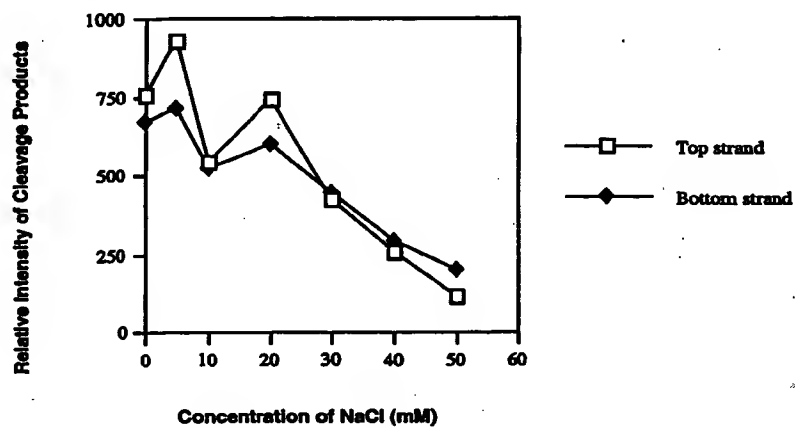


Figure 10

k-ras G12V (G→T)

A



B

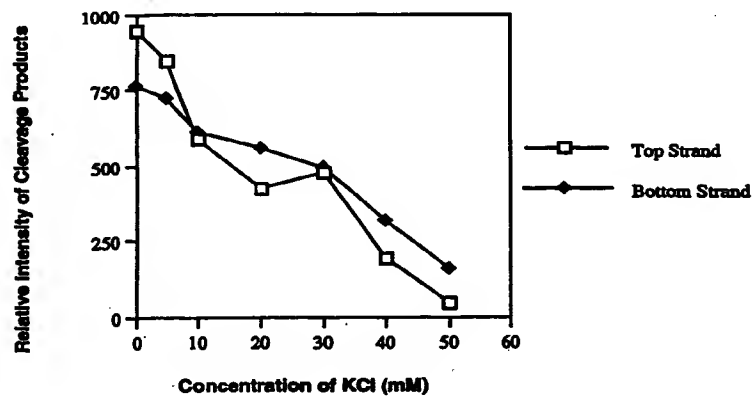


Figure 11

***k-ras* G12D (G→A)**

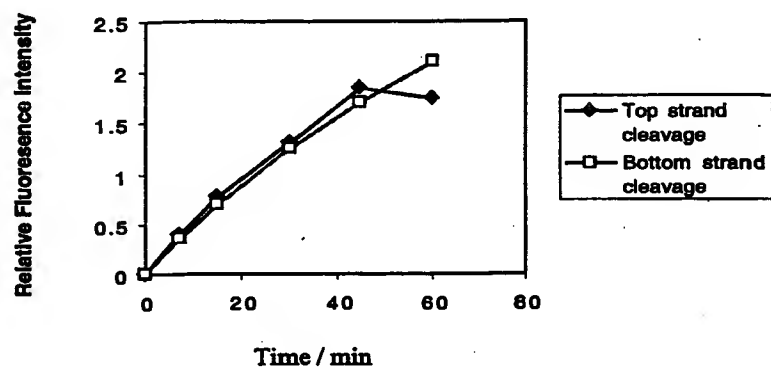


Figure 12

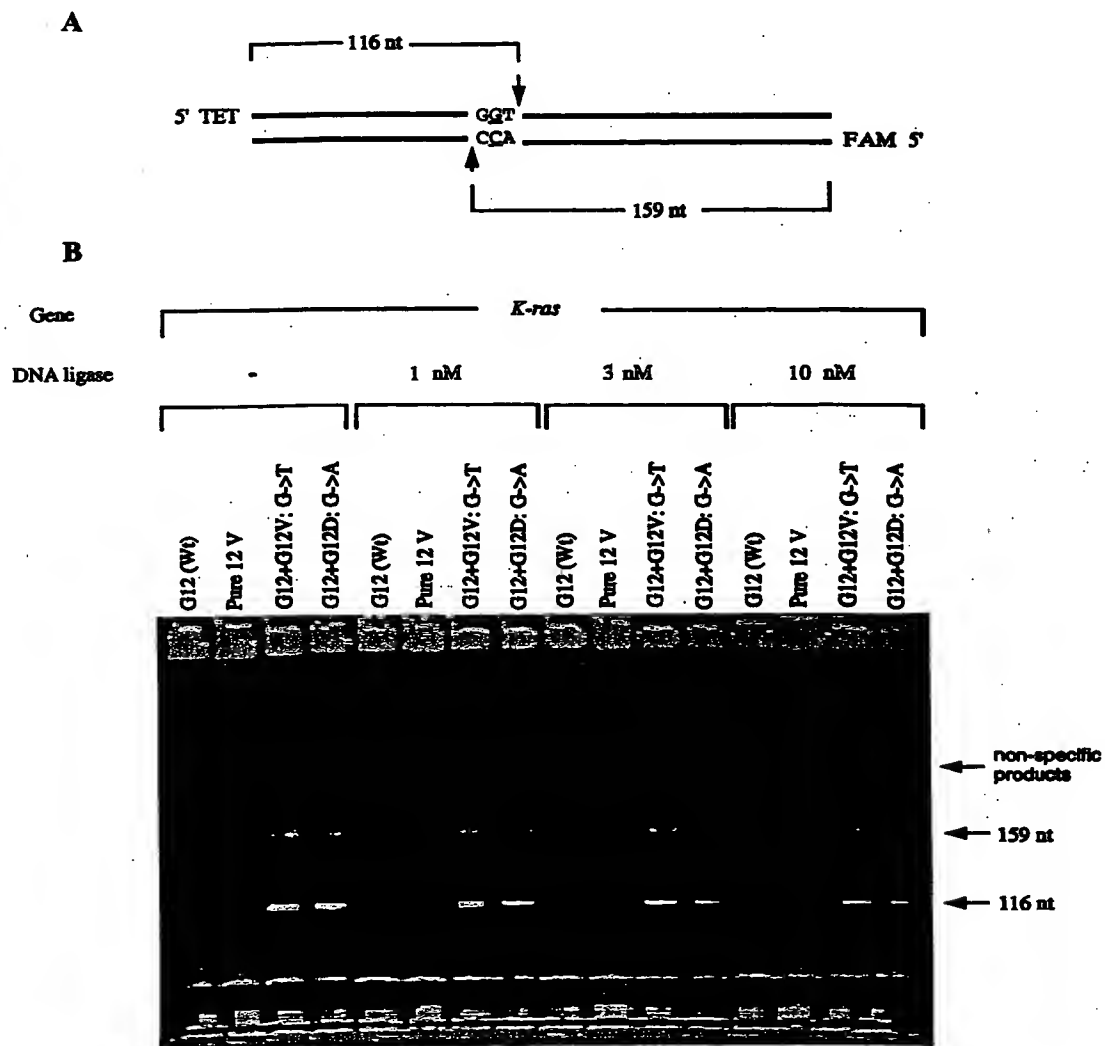


Figure 13

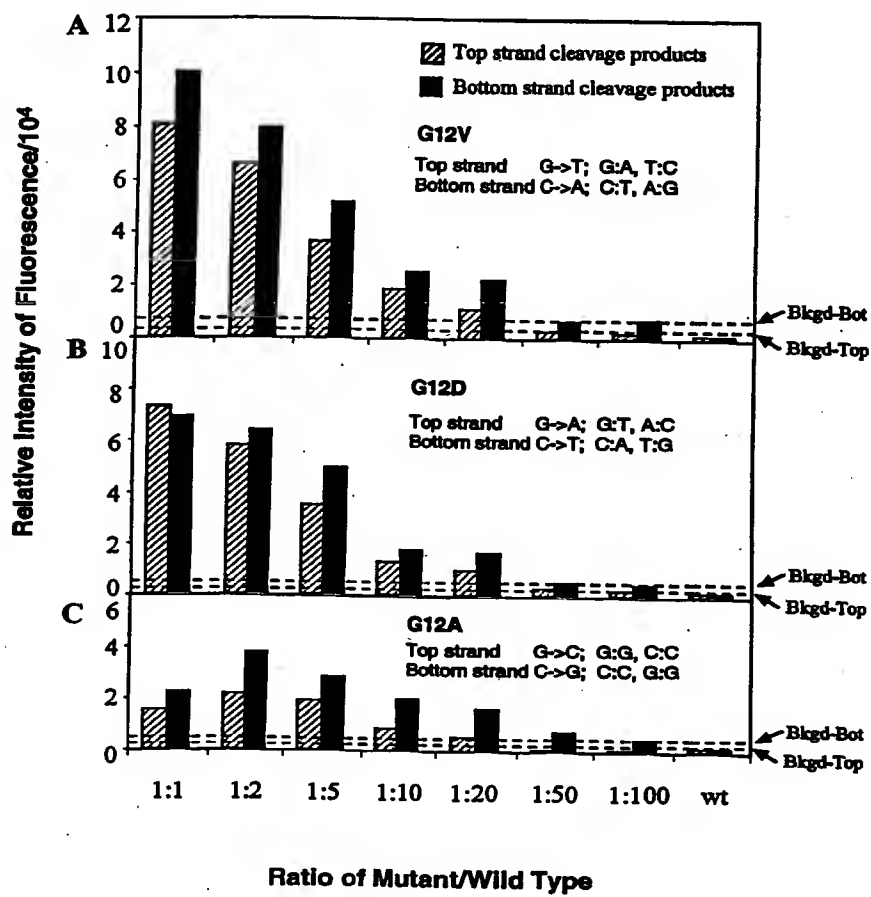


Figure 14

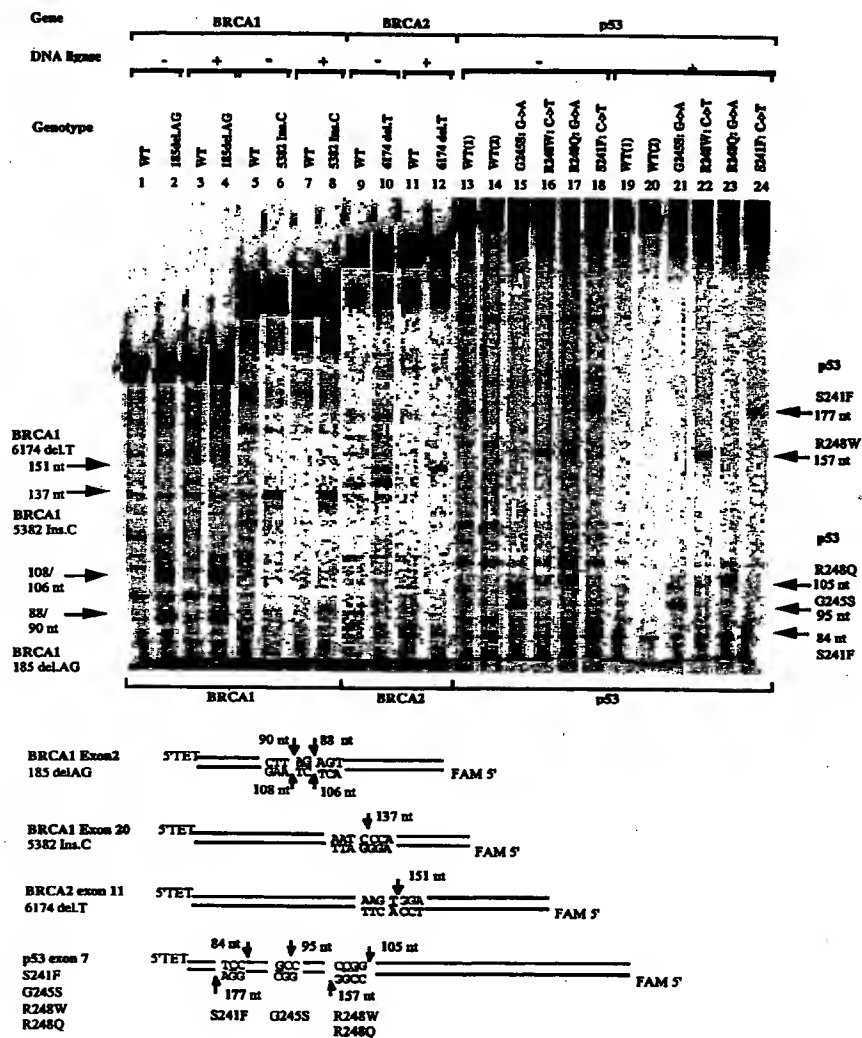


Figure 15

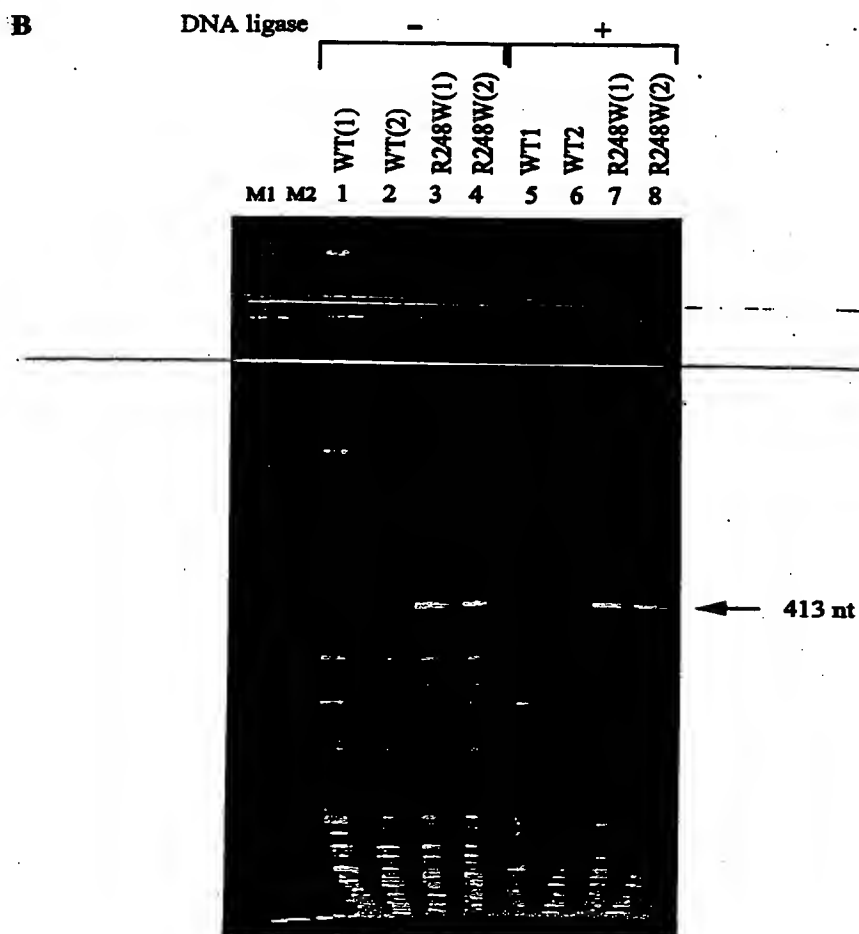
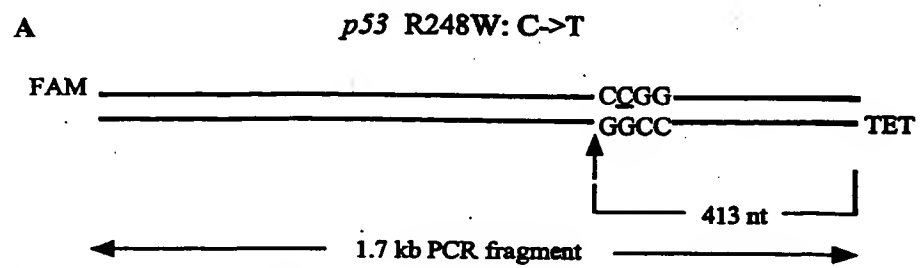
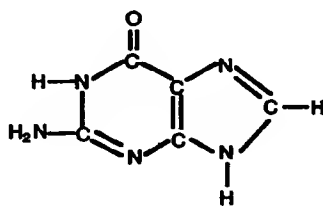


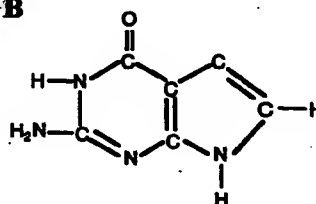
Figure 16

A



Guanine

B



7-deaza-Guanine

C

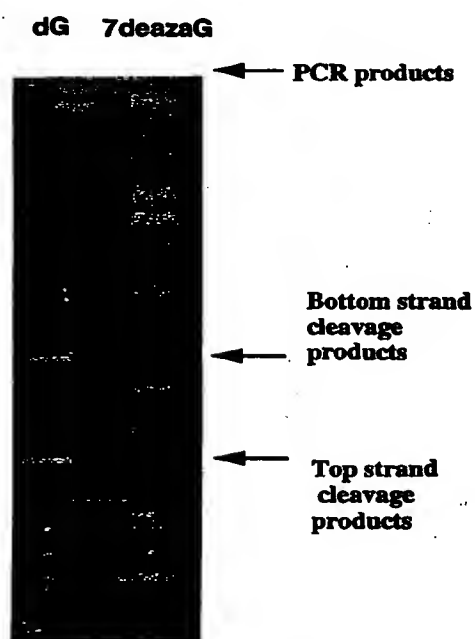


Figure 17

A

p53 R248W(C→T)

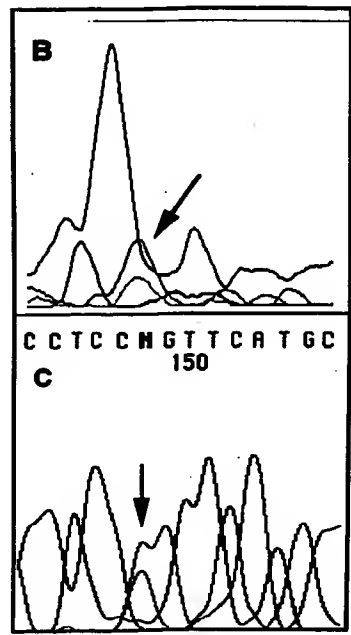
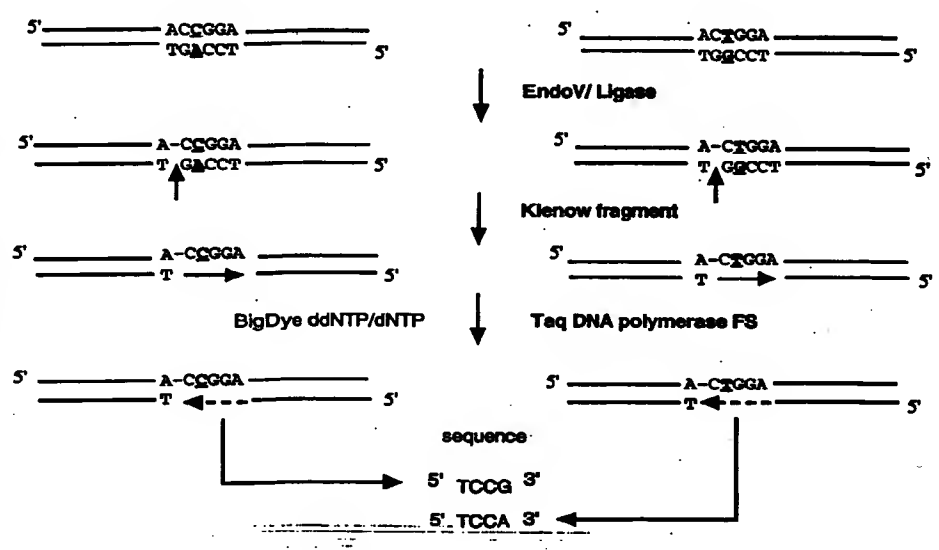


Figure 18

ML--DLA-----RAYQLAKSYLEED-I			Majority		
	19	20	30		
1	MDYRQLNR	VOLPP	DEATKYQNELRKKIKLTPY	Thermotoga maritima	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Pyrobaculum aerophilum	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Pyrobaculum horikoshii	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Pyrobaculum doylei	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Pyrobaculum furiosus	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Archaeoglobus fulgidus	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Aeropyrum pernix	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Clostridium acetobutylicum	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Therminia pepticus	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Escherichia coli	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Bacillus subtilis	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Salmonella typhimurium	
1	MRIIL	IFPRLPPIQITIA	QELKIKLTPY	Streptococcus coelicolor	

-YDEVELYAGVDAAC-----GGEYGRAAAVYLQYPSLEYE			Majority		
	49	50	60		
33	EGEPEYVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Thermotoga maritima	
41	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Pyrobaculum aerophilum	
21	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Pyrobaculum horikoshii	
21	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Pyrobaculum doylei	
25	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Pyrobaculum furiosus	
27	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Archaeoglobus fulgidus	
3	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Aeropyrum pernix	
25	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Clostridium acetobutylicum	
25	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Therminia pepticus	
26	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Escherichia coli	
35	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Bacillus subtilis	
24	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Salmonella typhimurium	
37	INEGRVAVAGV	PLS	PGKEGLAVIVLEYPSEKILE	Streptococcus coelicolor	

Block I

Figure 19

TKVAVGRVSPFIPGFLAF			LPPIAAATKKISEEPVYL			Majority		
70	80	90	100					
69	VYSEERGETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Thermotoga maritima		
74	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Pyrobaculum aerophilum		
56	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Pyrobaculum horikoshii		
56	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Pyrobaculum abyssi		
60	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Pyrobaculum furiosus		
63	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Archaeoglobus fulgidus		
32	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Aeropyrum pernix		
65	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Clostridium acetobutylicum		
63	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Yersinia pestis		
64	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Escherichia coli		
75	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Bacillus subtilis		
62	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Salmonella typhimurium		
75	IVLEETTF	IPGLLAF	GPFLKAEKLR	KPDVVV		Streptomyces coelicolor		

Block II

VYGHGIA			SRGLGLASHIGLLGKPTIGVAA			Majority		
120	130	140						
109	FQGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Thermotoga maritima		
114	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Pyrobaculum aerophilum		
94	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Pyrobaculum horikoshii		
94	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Pyrobaculum abyssi		
98	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Pyrobaculum furiosus		
103	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Archaeoglobus fulgidus		
72	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Aeropyrum pernix		
105	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Clostridium acetobutylicum		
103	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Yersinia pestis		
104	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Escherichia coli		
115	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Bacillus subtilis		
102	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Salmonella typhimurium		
115	FGGGGLA	PKLGIASHMGI	FIEIPTIGVAA	SRLYGT		Streptomyces coelicolor		

Block III

Block IV

Figure 19 (cont.)

F--LED--GA--PLLDGGEGLYVLRK-RCKPI--VSYG Majority		
150	159	170
146 FKNPEOKRCSVYLYGDEETIGCVIRKEGSAPI		VSPG
151		Thermoga mortua
152		Pyrobaculum aerophilum
153		Pyrococcus horikoshii
154		Pyrococcus abyssi
155		Pyrococcus furiosus
156		Archaeoglobus fulgidus
157		Aeropyrum pernix
158		Clostridium acetobutylicum
159		Yersinia pestis
160		Bacterioides coli
161		Bacillus subtilis
162		Salmonella typhimurium
163		Streptococcus coelicolor

Block V

HRLTDSALAIYQAL-ID--GYRLPEPTRLADALAK-R-- Majority		
190	200	210
185 HLMVSSKRLIKAFILP--GRRIPERTRLAH--ITYQRLK		220
186		Thermoga mortua
187		Pyrobaculum aerophilum
188		Pyrococcus horikoshii
189		Pyrococcus furiosus
190		Archaeoglobus fulgidus
191		Aeropyrum pernix
192		Clostridium acetobutylicum
193		Yersinia pestis
194		Bacterioides coli
195		Bacillus subtilis
196		Salmonella typhimurium
197		Streptococcus coelicolor

Block VI

Figure 19 (cont.)

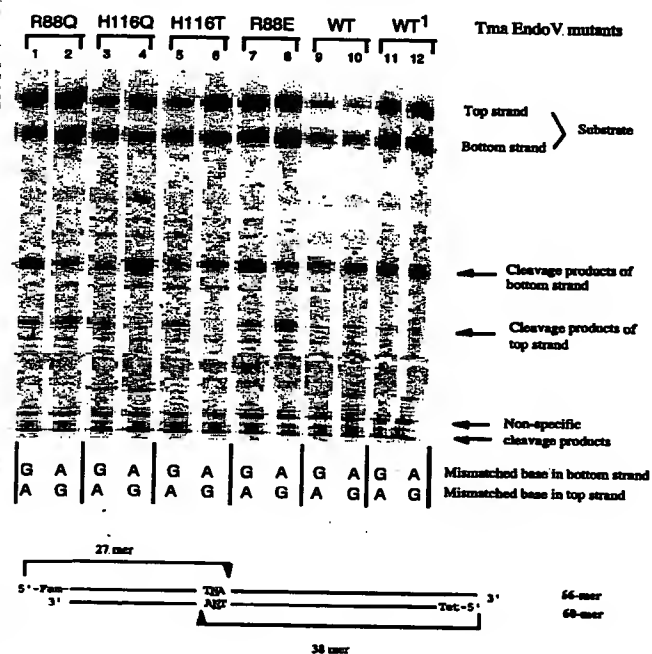


Figure 20

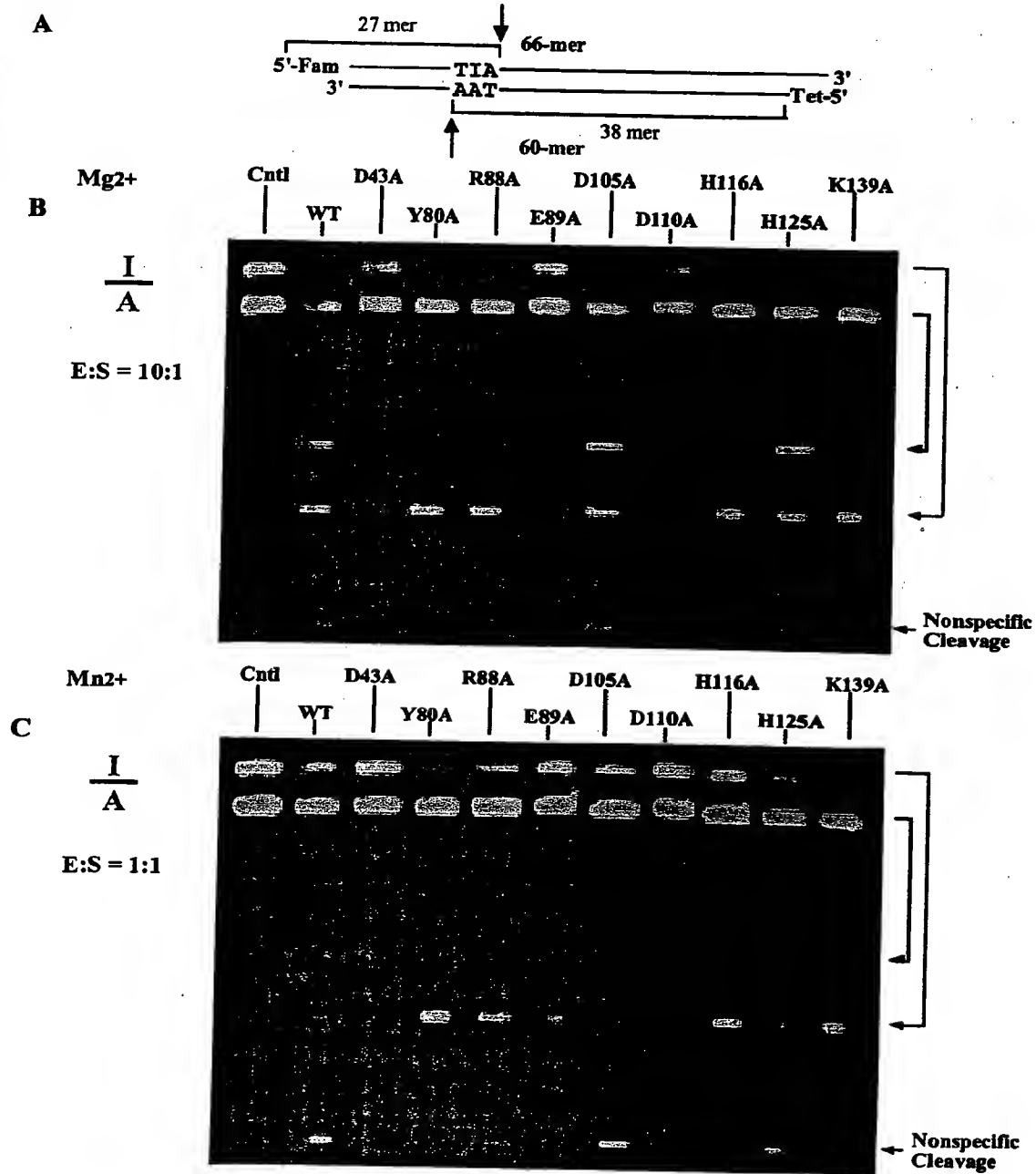


Figure 21

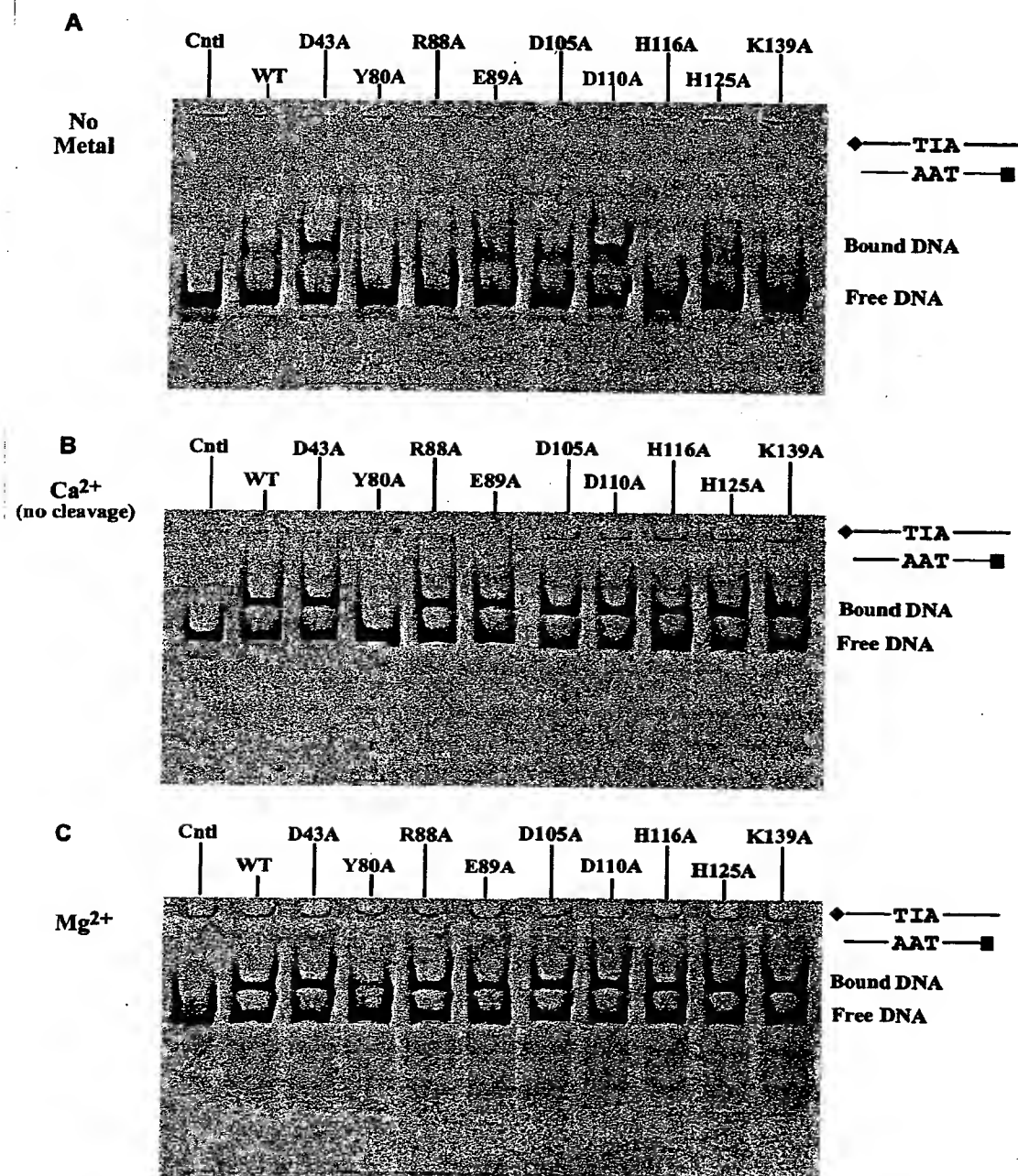


Figure 22

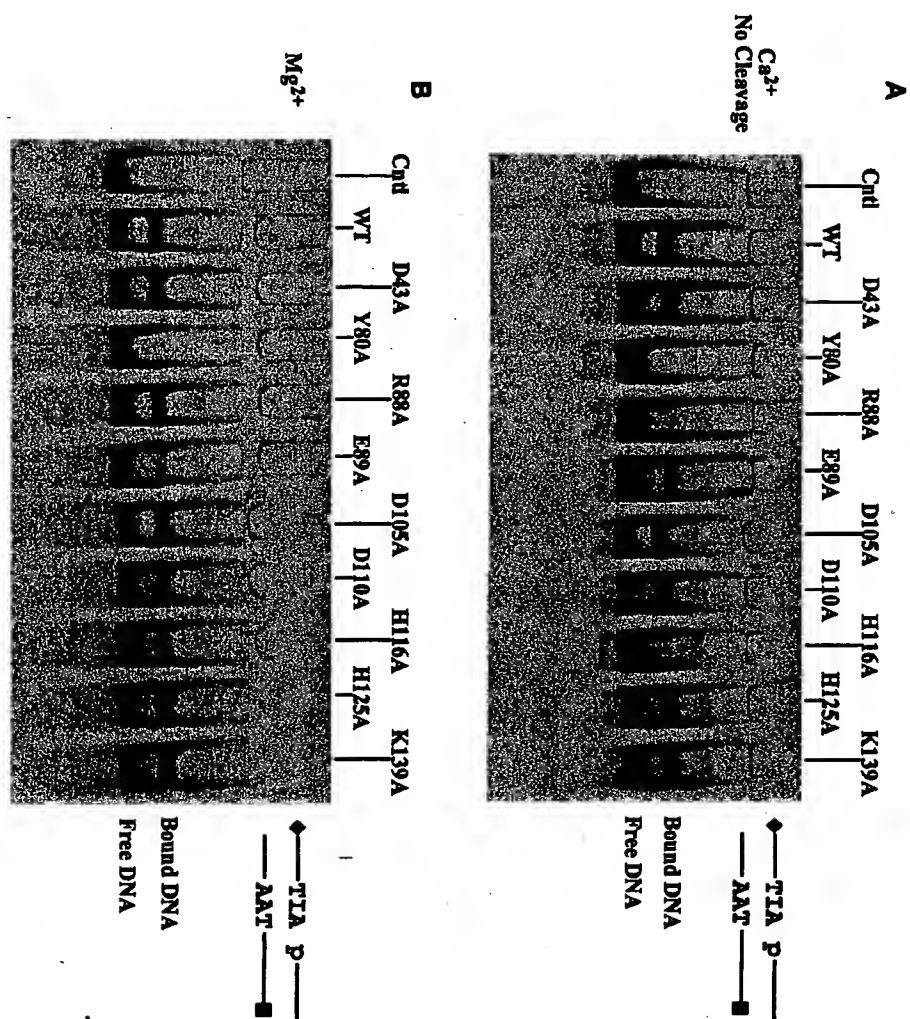


Figure 23

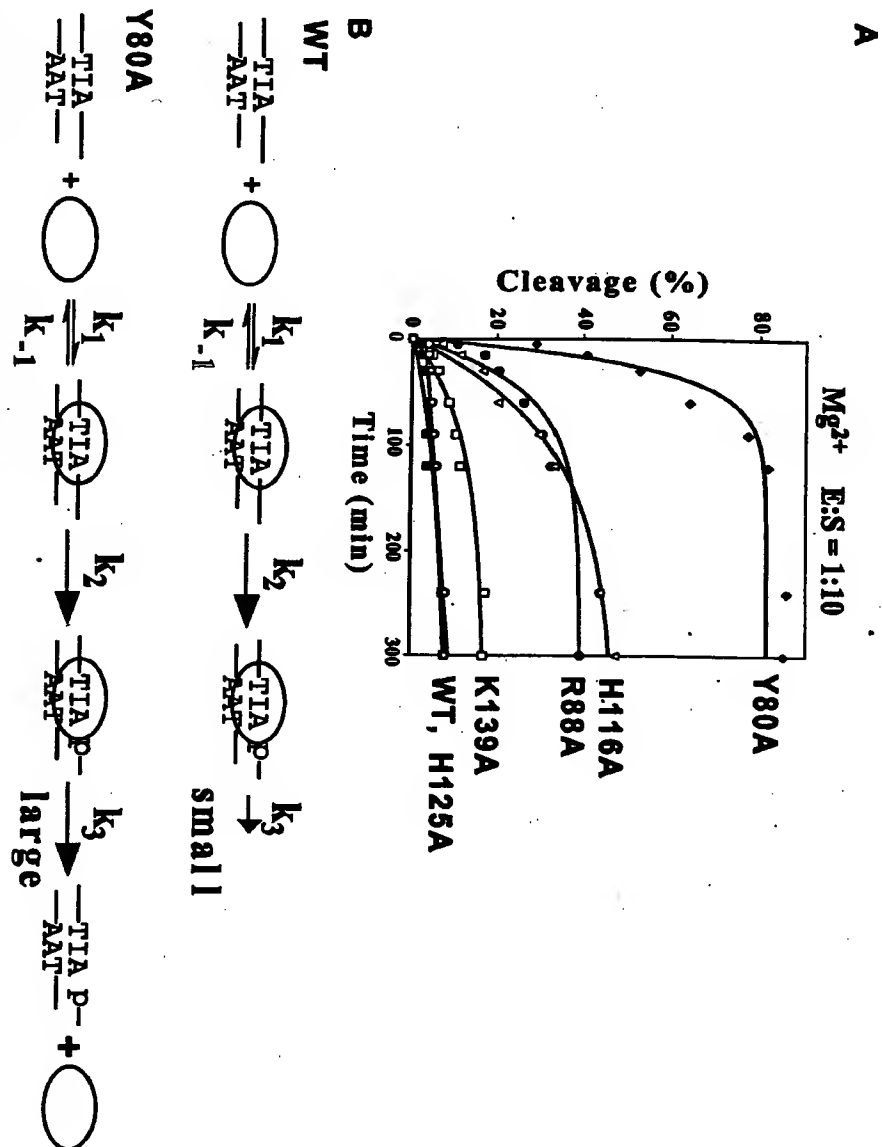


Figure 24

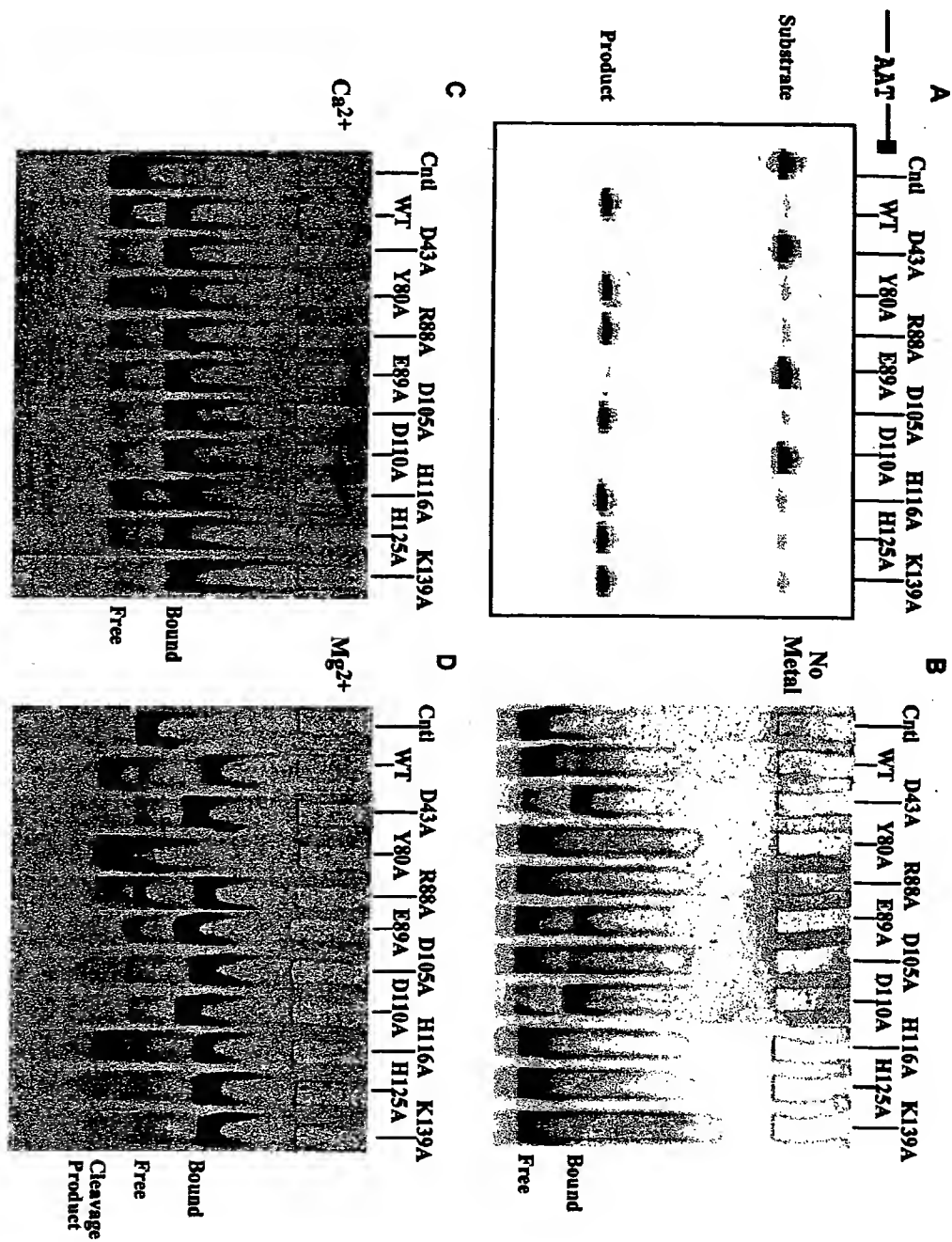


Figure 25

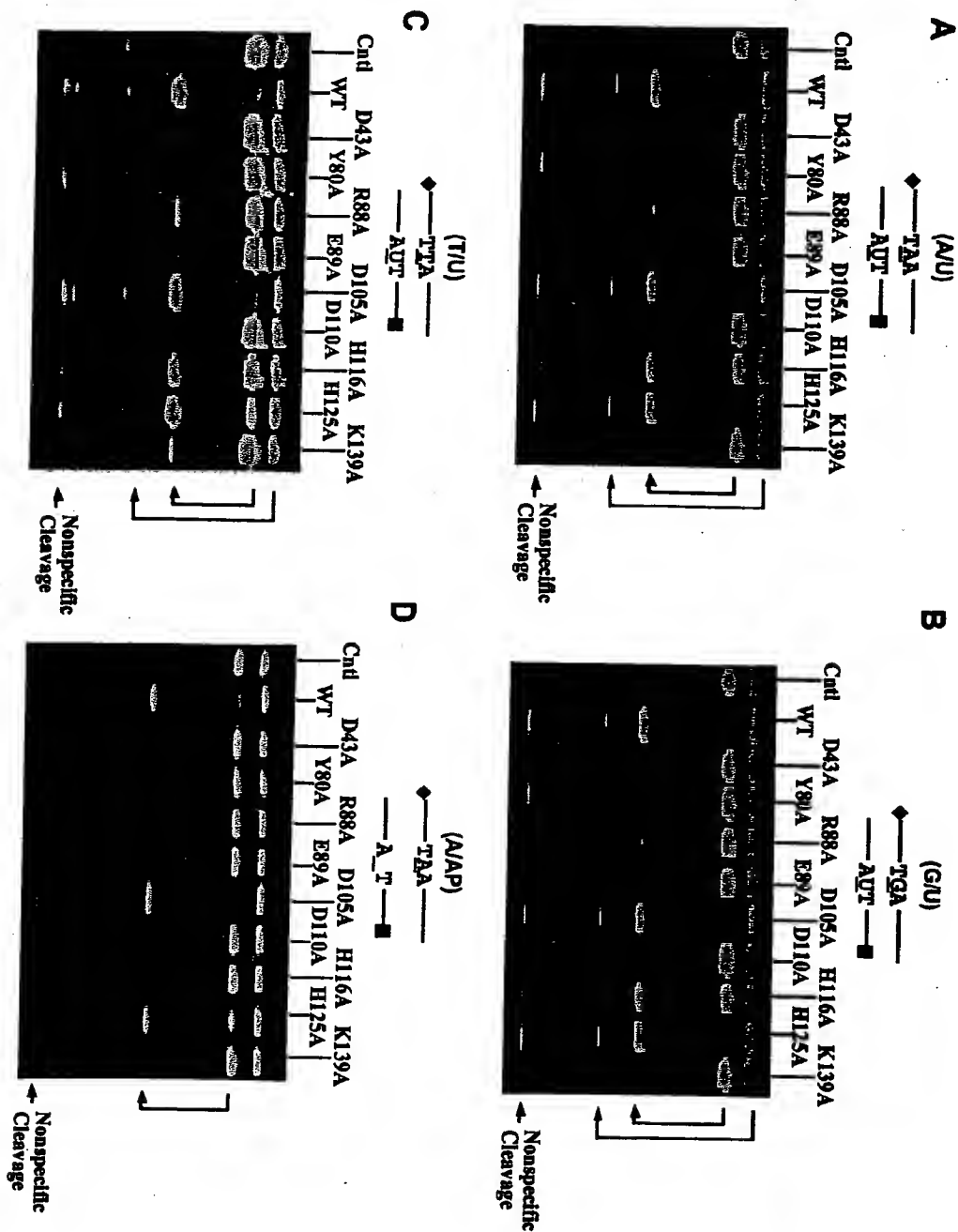


Figure 26